Reviewer's report

Title: Growth and metastasis of B16-F10 melanoma cells is not critically dependent on host CD73 expression in mice

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Reviewer: Linda Thompson

Reviewer's report:

General Comments:
This manuscript by Burghoff et al. describes the influence of host CD73 expression on the growth and metastasis of B16-F10 melanoma cells in mice. The work is similar to that published by Stagg et al. and Wang et al. in 2011 except that the Wang publication used B16-F10 melanoma cells expressing the model tumor antigen SIY. Both earlier papers showed that tumor growth was inhibited in CD73 KO mice. The Wang paper showed that both hematopoietic and non-hematopoietic CD73-expressing cells contributed to tumor progression. There were also increased numbers of CD8+ T cells in tumor infiltrates of tumor bearing CD73 KO mice. In contrast, Burghoff et al. found no contribution of CD73 to tumor growth or to the numbers of tumor infiltrating cells with the parental B16-F10 melanoma. Initial experiments were carried out with CD73 KO mice. It is unclear why the authors went to the trouble to repeat experiments in mice where CD73 was deficient in only endothelial cells or hematopoietic cells, given the negative results with mice in which CD73 was knocked out globally.

Discretionary Revisions:
1. The conditions for the ecto-5'-nucleotidase enzyme assay are not optimal. Normally, one would use a substrate concentration 10-fold higher than the Km. However, the concentration of substrate is only about 2-fold higher than the Km in this manuscript. Was the generation of substrate linear with time under these conditions?
2. The authors should show the data that reveal the efficiency of Tie2-Cre mediated deletion of the CD73 gene in endothelial cells.
3. The injection of B16-F10 melanoma cells i.v. is not really a model for metastasis.

Minor Essential Revisions:
1. The manuscript would benefit from being edited by a native English speaker. Some of the word choices do not follow standard English usage.
2. Figure 7 is described as being an IFN# ELISPOT on “tumor cells.” However, the assay was done on a mixture of tumor cells and infiltrating host leukocytes.
3. Were the tumors minced prior to enzymatic digestion?

Major Compulsory Revisions:
The authors conclude that their inability to see an effect of host CD73 deletion on B16-F10 melanoma cell growth is because of the low ecto-5’-nucleotidase on these cells. A more likely explanation may be that the B16-F10 melanoma is not very immunogenic. Deletion of CD73 slows tumor growth only in the presence of an intact immune system and is most convincing when the tumor cells express a model tumor antigen such as Ova or the SIY antigen in the case of B16-F10. In the earlier paper by Stagg et al. the growth of B16-F10 melanoma was only slightly decreased in CD73 KO mice – results which are not so different than those presented by Burghoff et al. The data on tumor infiltrating leukocytes are presented as % of CD45+ cells. If the data were presented in the same format as in the Wang paper, it would be easier to compare the results. There may be many fewer tumor infiltrating leukocytes with the parental B16-F10 cells.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.